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Copper Deficiency Decreases Plasma Homocysteine in Rats[1–4]

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Abstract

The purpose of this study was to determine the effects of copper deficiency on key aspects of homocysteine metabolism that involve methionine recycling and transsulfuration. Male weanling Sprague-Dawley rats were fed AIN-93G-based diets containing <1 or ~6 mg Cu/kg. After 6 wk (Expt. 1) and 4 wk (Expt. 2) we found that plasma homocysteine was significantly decreased, and plasma glutathione significantly increased, in rats fed the low-Cu diet. Real-time RT-PCR was used to determine the expression of the subunits of glutamate-cysteine ligase (Gcl) in liver that catalyzes the rate-limiting step in glutathione biosynthesis. The expression of Gclc, the catalytic subunit of Gcl, was upregulated by Cu deficiency; Gclm, the modifier subunit, was not affected. Hepatic betaine-homocysteine methyltransferase (Bhmt), which catalyzes one of the two ways that homocysteine can be remethylated to methionine, was downregulated by Cu deficiency. Because Cu deficiency results in upregulation of Gclc and an increase in the biosynthesis of glutathione, it is plausible that the net flux of homocysteine through the transsulfuration pathway is increased. Furthermore, if Bhmt is downregulated, less homocysteine is available for remethylation (methionine recycling) and more is then available to irreversibly enter the transsulfuration pathway where it is lost. The net effect of increased Gclc and decreased Bhmt would be a decrease in homocysteine as a result of Cu deficiency. J. Nutr. 137: 1370–1374, 2007.

Introduction

Copper (Cu) deficiency has long been recognized as targeting the cardiovascular system (1). However, the exact mechanism(s) of cardiac or cardiovascular involvement in Cu deficiency remains to be clearly identified (2). Over the years, research with laboratory animals has shown that Cu deficiency can result in cardiac defects (including changes in heart morphology and cardiac function), circulatory defects (including blood vessel morphology, angiogenesis, and circulatory function), and systemic defects (including changes in blood pressure and anemia) [for review see (1)].

Hyperhomocysteinemia is now regarded as a risk factor for cardiovascular disease (3). Brown and Strain (4) found that feeding rats supplemental DL-homocysteine lowered indices of Cu status; this occurred regardless of dietary Cu (adequate or deficient). However, until the work of Tamura et al. (5), nothing was known about the interaction between Cu deficiency and plasma concentrations of homocysteine; they reported that plasma homocysteine was elevated in Cu-deficient rats. Furthermore, Cu deficiency in rats results in increased plasma and liver glutathione (6). The increase in glutathione by Cu deficiency is most likely the result of an upregulation of γ-glutamylcysteine synthetase [glutamate-cysteine ligase (Gcl)[5]], the rate-limiting enzyme for glutathione biosynthesis (6). Work by Tamura et al. (5) suggested that homocysteine is increased as a result of decreased activity of methionine synthase (Mtr) in Cu-deficient rats. Contrary to the work of Tamura et al. (5), unpublished results from our laboratory indicate that plasma homocysteine is decreased, not increased, by Cu deficiency. Because of the well-known association that both homocysteine and Cu have with cardiovascular disease, it is important to understand any interaction between the two.

Our initial experiment was designed to determine the differences seen in Cu metabolism when the AIN-93 diet was compared with the AIN-76A diet (7). Reformulation of the AIN-76A diet included lowering the Mn concentration from 50 mg/kg to 10 mg/kg and changing the sulfur amino acid (SAA) supplement from DL-methionine (DL-Met) to L-cystine (L-Cys). Work by Kato et al. (8) and Nielsen (9) suggested that dietary cystine facilitates indirect defects of Cu deficiency by impairing the mobilization of Fe from the liver. Lowering the dietary Mn from 50 to 10 mg/kg diet might enhance Fe absorption thereby affecting signs of Cu deficiency (7). Also, an interaction between Cu and Mn exists that could affect Cu utilization (10,11). Thus, our experiment

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[5] Abbreviations used: Bhmt, betaine-homocysteine; Gcl, glutamate-cysteine ligase; Gclc, glutamate-cysteine ligase modifier subunit; Mtr, methyltetrahydrofolate-homocysteine methyltransferase; SAA, sulfur amino acid; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine.

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was designed to determine whether dietary Fe, Mn, and/or SAA interactions might influence the effect of Cu deficiency on plasma homocysteine. Because our results were contrary to the published results of Tamura et al. (5), who reported that Cu deficiency increases plasma homocysteine, we did not include our results on homocysteine in our report of the consequences on Cu as a result of alterations in the AIN-93 diet. Therefore, the purpose of this research was to verify that Cu deficiency lowers, not increases, plasma homocysteine, and to determine the effect of Cu deficiency on the mRNA expression of genes involved in homocysteine metabolism to determine the mechanism of action of Cu on homocysteine concentrations.

Methods and Materials

Animals and diets

Experiment 1. The experimental design is mentioned above and has already been published (7). To summarize, weanling male Sprague-Dawley rats ($n = 8$) were fed diets based on the AIN-93G diet (12). The experimental design was a $2 \times 2 \times 2 \times 2$ factorial in which the dietary variables were Cu, <1 or 6 mg/kg; Fe, 10 or 35 mg/kg; Mn, 10 or 50 mg/kg, and SAA as either DL-Met or L-Cys, each at 3 g/kg. The diets contained 200 g casein/kg. Thus, the L-Cys–supplemented diets contained (by calculation) 3.5 g cystine and 4.6 g methionine/kg; the DL-Met-supplemented diets contained 0.5 g cystine and 7.6 g methionine/kg. Rats were fed the diets for 6 wk and then, without fasting, were anesthetized with a 1.37:1 mixture of ketamine:xylazine (1 mL/kg body wt, i.p.) (Ketamine HCl, Fort Dodge Animal Health; Xylazine, Phoenix Scientific). Blood was withdrawn from the abdominal aorta into tubes containing EDTA (30 μL of 121 mmol/L EDTA, diNa, pH 7.4, per mL whole blood) and centrifuged at $10,000 \times g$ at 4°C within 30 min.

Experiment 2. Male weanling Sprague-Dawley rats ($n = 8$) were fed AIN-93G-based diets (12) with Cu as the dietary variable (<1 or 6 mg/kg). After 4 wk unfasted rats were anesthetized with fluorescene. EDTA-blood was collected and centrifuged at $10,000 \times g$ at 4°C within 30 min. The analyzed Cu concentrations of the diets were 0.30 ± 0.01 mg/kg for the <1 mg/kg Cu diet and 6.38 ± 0.11 mg/kg for the 6 mg/kg Cu diet. Cu was supplemented as $CuSO_4 \cdot 5H_2O$.

All procedures were approved by the Animal Care and Use Committee of the USDA Agricultural Research Service, Grand Forks Human Nutrition Research Center; the study was in accordance with the guidelines of the NIH on experimental use of laboratory animals (13).

Cu status indices

Plasma ceruloplasmin activity, Cu content of the diets, and liver Cu and Fe concentrations were determined as reported (7).

Liver SAM and SAH

Liver was homogenized in cold 0.4 mol/L perchloric acid and prepared for *S*-adenosylmethionine (SAM) and *S*-adenosylhomocysteine (SAH) analysis according to Davis et al. (14) and measured with a Dionex 4000i HPLC (Dionex) according to the procedure of Wagner et al. (15).

Plasma homocysteine, glutathione, and cysteine

Total (reduced plus oxidized) homocysteine, total glutathione, and cysteine were determined in EDTA plasma using HPLC according to the procedure of Durand et al. (16).

Real time RT-PCR

During the animal kill a piece of liver was quickly excised and placed in RNAlater (Ambion) and then stored at −20°C until RNA extraction. Total RNA was extracted using the NucleoSpin RNA II kit (BD Biosciences). The primers used for the different genes were: Bhmt (betaine-homocysteine methyltransferase, NM_030850), F: 5′-GGG AAC TAC GTG GCA GAG AA-3′, R: 5′-CGT GCA ATG TCA CAA GCA G-3′; Gclc (glutamate-cysteine ligase, NM_012815), F: 5′-GGC AAG ATA CCT TTA TGA CCA GTT-3′, R: 5′-TGC AGC ACT CAA AGC CAT AA-3′; Gclm (glutamate-cysteine ligase, modifier subunit, NM_017305), F: 5′-AGA CAA AAC ACA GTT GGA GCA G-3′, R: 5′-CAG TCA AAT CTG GTG GCA TC-3′; Mtr (5-methyltetrahydrofolate-homocysteine methyltransferase, NM_030864), F: 5′-AGC CGC CGA GGA GAT AAC-3′, R: 5′-TAG TCG GAC CCA GAG ATC CA-3′; Actb (β-actin, NM_031144), F: 5′-CCC GCG AGT ACA ACC TTC T-3′, R: 5′-CGT CAT CCA TGG CGA ACT-3′; Mat2a (methionine adenosyltransferase II α, NM_134351), F: 5′-ATG CTG TCC TTG ATG CAC AC-3′, R: 5′-TTC CAG TTT TAG CAA CAG TTT CAC-3′; Mthfr (5,10-methylenetetrahydrofolate reductase, XM_342975), F: 5′-GGA GGT GGT AGA GGC TGG T-3′, R: 5′-TGA AGC TTG TCT GGA TGG AGT-3′; Gnmt (glycine *N*-methyltransferase, NM_017084), F: 5′-ACT GAA GGA GCG CTG GAA C-3′, R: 5′-CAG TTG GCT TCT TCA ATG ACC-3′; Ahcy (*S*-adenosylhomocysteine hydrolase, NM_017201), F: 5′-GTT CAC TTC CTG CCT AAG AAG C-3′, R: 5′-AGC TTG ACG TTC AGC TTG C-3′.

Real time RT-PCR was done using the QuantiTect SYBR Green RT-PCR kit (Qiagen) and a SmartCycler (Cepheid) instrument. Gene expression was quantified using the comparative C_T method (17). C_T, the threshold cycle, is the number of cycles it takes for a sample to reach the level where the rate of amplification is greatest during the exponential phase. ΔC_T was obtained for each sample or gene by the following calculation: $\Delta C_T = C_{T,X} - C_{T,R}$ where $C_{T,X}$ = threshold number for the target gene amplification and $C_{T,R}$ = threshold number for the reference gene [β-actin (Actb)] amplification. For the comparative C_T method to be valid, replication efficiencies (slope of the calibration curves) of the target gene and that of the reference gene must be approximately equal. For the primers used, replication efficiencies were equal to the efficiency of the reference amplification (Actb) (data not shown).

The amount of target, normalized to an endogenous reference (Actb) and relative to the control group (rats fed 6 mg Cu/kg), is given by the formula $2_T^{-\Delta\Delta C}$; $\Delta\Delta C_T = \Delta C_{T,q} - \Delta C_{T,cb}$ where $\Delta C_{T,q} = \Delta C_T$ for an individual sample and $\Delta C_{T,cb}$ = mean ΔC_T for control group. Thus, the value of $2_T^{-\Delta\Delta C}$ for the amount of target in the control group was = 1. For the amount of target in the animals fed <1 Cu mg/kg, a 1-fold change indicates no change, >1-fold change indicates upregulation, and <1-fold change indicates downregulation.

Statistics

Data from Expt. 1 were analyzed by 4-way ANOVA using the statistical package SAS, version 9.1 (SAS Institute) in which the main effects and interactions were computed. The *t* test (Excel, Microsoft) was used for data from Experiment 2. For the real-time RT-PCR data, the *t* test compared ΔC_T. However, fold change ($2_T^{-\Delta\Delta C}$) is reported in the text. All values are reported as means ± SE or, for fold change, means are expressed as (−1 SE, +1 SE). Differences were considered significant at $P \leq 0.05$.

Results

Data on Cu status indices for Expt. 1 have been reported (7). Cu-deficient rats over a range of Fe, Mn, and SAA intakes, have decreased concentrations of plasma homocysteine compared with rats fed adequate copper (**Table 1**, $P < 0.0008$). Only in Cu-deficient rats, fed 35 mg/kg Zn, 50 mg/kg Mn, and L-Cys, was plasma homocysteine higher than in rats fed the same diet but supplemented with Cu. Of the other dietary variables, only Fe significantly affected plasma homocysteine (Table 1). Rats fed low dietary Fe (10 mg/kg) had reduced plasma homocysteine than those fed 35 mg Fe/kg.

Dietary Cu had no direct effect on the concentration of hepatic SAM (Table 1). Liver SAH was slightly ($P = 0.023$) elevated by Cu deficiency. Hepatic SAH was increased, and the SAM:SAH ratio decreased, in rats fed diets containing 10 mg Fe/kg.

Because the main objective of this research was to determine the effect of Cu on homocysteine and homocysteine metabolism, we included the results when all dietary treatment groups (Fe, Mn, and SSA) other than Cu were pooled (**Table 2**). Compared

TABLE 1 The effect in rats of dietary Cu, Fe, Mn, and sulfur amino acid on the concentrations of plasma homocysteine, SAM, SAH, and the ratio of SAM:SAH (Expt. 1)

Treatment				Plasma	Liver		
Cu	Fe	Mn	SAA[1]	homocysteine	SAM	SAH	SAM:SAH
mg/kg				*μmol/L*	*nmol/g*		
6	35	10	L-Cys	8.37 ± 1.02[2]	63.9 ± 2.0	19.5 ± 1.2	3.35 ± 0.21
6	35	10	DL-Met	7.44 ± 0.64	66.4 ± 1.7	19.6 ± 0.9	3.46 ± 0.23
6	35	50	L-Cys	6.00 ± 0.69	74.9 ± 3.1	19.7 ± 1.4	3.87 ± 0.15
6	35	50	DL-Met	7.56 ± 0.88	70.6 ± 4.7	20.5 ± 1.1	3.51 ± 0.27
6	10	10	L-Cys	5.35 ± 0.52	67.7 ± 3.8	21.4 ± 0.6	3.18 ± 0.18
6	10	10	DL-Met	5.63 ± 1.10	66.5 ± 3.2	21.0 ± 0.8	3.19 ± 0.17
6	10	50	L-Cys	6.02 ± 0.68	66.5 ± 2.8	22.3 ± 0.6	3.00 ± 0.15
6	10	50	DL-Met	4.73 ± 0.63	65.2 ± 3.7	20.3 ± 0.8	3.23 ± 0.14
<1	35	10	L-Cys	5.77 ± 0.92	70.7 ± 3.7	19.4 ± 0.9	3.67 ± 0.21
<1	35	10	DL-Met	5.06 ± 0.44	76.4 ± 3.8	22.8 ± 0.8	3.53 ± 0.18
<1	35	50	L-Cys	7.03 ± 0.67	63.3 ± 3.5	21.8 ± 0.8	2.91 ± 0.11
<1	35	50	DL-Met	4.66 ± 0.58	68.5 ± 4.2	21.0 ± 1.0	3.32 ± 0.24
<1	10	10	L-Cys	5.29 ± 0.57	68.6 ± 5.5	23.1 ± 0.9	2.98 ± 0.23
<1	10	10	DL-Met	4.14 ± 0.44	68.3 ± 3.6	21.4 ± 0.9	3.20 ± 0.12
<1	10	50	L-Cys	4.97 ± 0.72	68.2 ± 4.1	21.3 ± 1.0	3.27 ± 0.29
<1	10	50	DL-Met	4.38 ± 0.36	69.9 ± 4.0	22.1 ± 0.9	3.21 ± 0.24
ANOVA[3]					*P-value*		
Cu				0.0008	—[4]	0.023	—
Fe				0.0001		0.024	0.004
Cu × Fe × Mn				—	0.022	—	0.015
Cu × Fe × Mn × SAA				0.028	—	0.018	—

[1] The diet was supplemented with 0.3% of either DL-methionine (DL-Met) or L-cystine (L-Cys).
[2] Values are means ± SEM, $n = 7$–8.
[3] The following main effects and interactions were not significant and hence were not included in the table: Mn, SAA, Cu × Fe, Cu × Mn, Cu × SAA, Fe × Mn, Fe × SAA, Mn × SAA, Cu × Fe × SAA, Cu × Mn × SAA, Fe × Mn × SAA.
[4] $P > 0.05$.

with rats fed 6 mg Cu/kg, Cu deficiency resulted in ~20% reduction in plasma homocysteine.

The Cu-status indicators show that the rats from Experiment 2 fed the low-Cu diet were Cu deficient (**Table 3**).Plasma ceruloplasmin activity and liver Cu were markedly depressed ($P < 0.0001$), and liver Fe elevated ($P < 0.001$), by Cu deficiency.

Plasma homocysteine, but not cysteine, was decreased by Cu deficiency (**Table 4**). Plasma glutathione was increased in rats fed the Cu deficient diet.

Expression of genes related to homocysteine metabolism was determined by real time RT-PCR. Of 8 genes studied, only Gclc and Bhmt were affected by Cu. Gclc was upregulated [2.86-fold increase (1.78, 4.59)] in the Cu-deficient rats compared with the Cu-adequate controls ($P < 0.04$). Bhmt was downregulated [0.48-fold decrease (0.36, 0.65)] in liver of rats fed the Cu-deficient diet compared with controls fed 6 mg Cu/kg ($P < 0.024$).

Discussion

Copper status indicators of Experiment 1 have been published (7). For example, the activity of serum ceruloplasmin was reduced ($P < 0.001$) in Cu deficiency to only ~5% of the control values. Plasma and liver Cu concentrations and plasma homocysteine were markedly reduced, and liver Fe markedly increased, by Cu deficiency. The decrease in plasma homocysteine due to Cu deficiency occurred under a variety of dietary conditions including altered Fe, Mn, and SAA. However, the scope of the present research was only to determine the effect of Cu deficiency on homocysteine. Therefore, we pooled dietary groups to show the overall effect of Cu (Table 2). Because these results contradicted findings of Tamura et al. (5), our second experiment included efforts to not only study the effect of Cu on the concentration of homocysteine in plasma, but to study the effects of Cu on the metabolism of homocysteine as well.

TABLE 2 Effect in rats of dietary Cu on the concentrations of plasma homocysteine, SAM, SAH, and the ratio of SAM:SAH (Expt. 1)

Treatment[1]	Homocysteine	SAM	SAH	SAM:SAH
mg/kg	*μmol/L*	*nmol/g*		
<1	5.16 ± 0.25[2]	69.2 ± 1.3	21.6 ± 0.33	3.26 ± 0.072
6	6.39 ± 0.25	67.7 ± 1.3	20.5 ± 0.33	3.35 ± 0.071
P-value	0.0008	—[3]	0.023	—

[1] Diets grouped by Cu only (regardless of Fe, Mn, or SAA).
[2] Values are means ± SEM, $n = 62$–64.
[3] $P > 0.05$.

TABLE 3 The effect in rats of dietary Cu on the Cu-status indices of ceruloplasmin, liver Cu, and liver Fe (Expt. 2)

Cu treatment	Ceruloplasmin	Liver Cu	Liver Fe
mg/kg	*U/L*	*μmol/kg dry wt*	*mmol/kg dry wt*
<1	0.53 ± 0.14[1]	30.1 ± 5.6	4.4 ± 0.3
6	95.6 ± 6.8	175 ± 6.4	2.7 ± 0.2
P-value[2]	<0.0001	<0.0001	<0.001

[1] Values are means ± SEM, $n = 7$–8.
[2] *P*-values determined by *t* test.

TABLE 4 Effects in rats of dietary Cu on plasma homocysteine, cysteine, and glutathione (Expt. 2)

	Plasma		
Treatment, *Cu mg/kg*	Homocysteine	Cysteine	GSH
		μmol/L	
<1	4.05 ± 0.27[1]	65.7 ± 3.4	11.1 ± 1.5
6	5.28 ± 0.48	67.8 ± 2.0	6.7 ± 0.3
P-value	< 0.040	—[2]	< 0.013

[1] Values are means ± SEM, $n = 7$–8.
[2] $P > 0.05$.

Experiment 2 confirmed that Cu deficiency resulted in a significantly decreased concentration of plasma homocysteine. Further, we showed that plasma glutathione is increased in Cu deficiency. Cu deficiency in rats increases plasma and hepatic glutathione (18). To determine why Cu deficiency increases glutathione concentrations, Chen et al. (6) studied the activity and mRNA expression of hepatic γ-glutamylcysteine synthetase (glutamate-cysteine ligase, Gcl). They found that the activity of Gcl and the relative amount of Gcl mRNA were significantly increased by Cu deficiency. Because this enzyme catalyzes the rate limiting step of glutathione synthesis, their work provided a mechanism for increased glutathione in Cu-deficient animals. Our work verified that Cu deficiency results in upregulation of Gcl mRNA. We also found that Cu deficiency upregulates Gclc mRNA, the catalytic subunit of Gcl, but we did not find an effect of Cu on Gclm mRNA, the modifier subunit of Gcl. Of other genes involved in homocysteine metabolism that we selected to analyze for expression, only Bhmt was affected by Cu deficiency. Bhmt mRNA was significantly downregulated in the Cu-deficient rat liver. The enzyme expressed by Bhmt is important for remethylation of homocysteine to methionine, deriving the methyl group from betaine. The other enzyme that remethylates homocysteine to methionine is the folate-dependent methionine synthase (Mtr). Tamura et al. (5) found that the activity of hepatic Mtr was decreased by Cu deficiency. We found no effect of Cu on the expression of Mtr (mRNA).

Although Cu deficiency resulted in a slight increase (~5%) in liver SAH (Table 2), it was significant. SAH is hydrolyzed to form homocysteine and adenosine by the gene product (SAH hyrdolase) of Ahcy. However, because of the thermodynamics of the SAH hydrolase reaction, SAH concentrations usually reflect the tissue concentration of homocysteine (19). Furthermore, SAH hydrolase is reported to be a Cu-binding protein (20). However, we found that dietary Cu had no effect on the expression of Ahcy mRNA. Also, Tamura et al. (5) stated "Our preliminary results of the determination of the activities of SAH hydrolase in the liver and red cells of the rats used in the study presented here indicate that SAH hydrolase activities were not decreased in copper-deficient rats." We also found no effect of Cu on SAM or SAM:SAH ratio. Thus, it was not unexpected that we found no effect of Cu on Gnmt mRNA, the gene product of which plays an important role in regulating tissue SAM and SAH and therefore SAM:SAH ratio (21,22).

Cu deficiency results in upregulation of Gclc mRNA and an increase in the biosynthesis of glutathione. Mosharov et al. (23) suggest that about half of the intracellular glutathione pool in human liver cells is derived from homocysteine. Thus, it is not unreasonable to suggest that upregulation of Gclc results in decreased homocysteine in Cu-deprived rats. Furthermore, if Bhmt is downregulated, less homocysteine is available for remethylation (methionine recycling) and more is then available to irreversibly enter the transsulfuration pathway where it is lost.

Hyperhomocysteinemia is now regarded as a risk factor for atherosclerosis (24). Therefore, it is important to know how dietary factors can affect its metabolism and ultimate concentration. Tamura et al. (5) found that plasma homocysteine was increased by Cu deficiency. We had opposite findings, that Cu deficiency results in a decreased concentration of plasma homocysteine. This does not suggest that the effect of copper deficiency on homocysteine is beneficial. Our results suggest that an impairment in metabolism as a result of copper deficiency results in the lowering of homocysteine to below normal steady-state concentrations. The decrease in homocysteine may actually be detrimental, in that Dudman (25) suggested that homocysteine may be needed for recruitment of leucocytes at sites of tissue damage.

Our results occurred in studies with overtly Cu-deficient rats; the impact in laboratory animals and humans of marginal deficiency of Cu on homocysteine is not known. Overt copper deficiency does not occur in humans. However, Milne reported that >30% of 849 diets sampled from North America and Europe provided <1.0 mg Cu/d, the approximate amount of Cu found to be insufficient in some short-term Cu-depletion studies in humans (26).

We have no explanation for the differences between our findings and those of Tamura et al. (5). However, they could be the result of differences in diets or age of animals at the onset of the experiments. Both groups used male Sprague-Dawley rats. We used weanling rats (~40 g) but Tamura used older rats of ~90 g. Both groups used approximately the same time (our studies were 6 and 4 wk; Tamura et al. used 6 wk). The main difference was the diets used. We used the 20% casein-based AIN-93 diet (12) but Tamura et al. (5) used a 21% egg-white based diet. This would mean (based on calculated values) that the molar ratio (expressed either as cystine:methionine or methionine:cystine) were significantly different between the studies (regardless of methionine or cystine supplementation in our first experiment). Other amino acid differences included more glycine and serine in the egg white diets. All of these amino acids can impact methionine and hence homocysteine metabolism (27–29). Also, Fields et al. (30) showed that the type of dietary protein can affect signs of Cu deficiency.

Finally, the effect of Cu deficiency on glutathione, homocysteine, and Gclc mRNA parallels that of selenium deficiency in rats. In a selenium-deficient rat, there is low plasma (and tissue) homocysteine, increased glutathione (hepatic and plasma) (31), decreased activity and expression of Bhmt (32), and upregulation of Gcl (33). Both Cu and selenium are involved in oxidative defense, whether mechanisms resulting in similar outcomes related to homocysteine and homocysteine metabolism are the same, is unknown.

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